HOECHST AKTIENGESELLSCHAFT HOE 97/F006 Dr. OL/we

Description

Gene constructs for active substances which can be activated by proteases

1) Introduction

Like inflamed areas, tumors are distinguished from the surrounding normal tissue by a substantial increase in the formation and secretion of proteases [review in Schmitt et al., Fibrinol. 6, 3 (1992), Cottam et al., Int. J. Oncol. 2, 861 (1993), Tryggvason et al., Breast Cancer Res. And Treatm. 24, 209 (1993), Leto et al., Anticancer Res. 12, 235 (1992), Hart, Fibrinol. 6, 11 (1992) and Albini et al., J. Natl. Cancer Inst. 83, 735 (1991)]. Examples of these proteases are plasminogen activators, cathepsins and matrix metalloproteinases.

An essential function of these tumor proteases is to dissolve the extracellular matrix to allow the tumor cells to invade, and grow in an infiltrative manner in, normal tissue.

At the same time, these proteases protect the tumor from the defense mechanisms of the body in as far as the active compounds which are required for defense are cleaved, and thereby inactivated, by the proteases which are formed by the tumor. Thus, for example, antibodies, cytokines and growth factors, complement factors, coagulation factors and mediators are inactivated by tumor proteases.

Knowing these protective mechanisms, the aim of many research groups has been to inhibit the infiltrative and metastatic growth of tumors, and the inactivation of the defense mechanisms of the body, by inhibiting the tumor

cell proteases [reviews in Hocman, Int. J. Biochem. 24, 1365 (1992), Troll et al., JNCI 73, 1245 (1984), Ray et al., Eur. Respir. 7, 2062 (1994), Koop et al., Cancer Res. 54, 4791 (1994), Chiriri et al., Int. J. Cancer 58, 460 (1994), Denhardt et al., 59, 329 (1993) and Melchiori et al., Cancer Res. 52, 2353 (1992)].

However, this experimental approach has so far had little success, usually for stoichiometric and pharmacokinetic reasons.

Instead of inhibiting tumor cell proteases, therefore, an attempt was made to use them to activate bacterial toxins such as Staphylococcus aureus α-hemolysin [Panchal et al., Nature Biotechn. 14, 852 (1996)]. For this, insertion of an amino acid sequence XX-Arg-X into positions 129 to 132 of the α-hemolysin was used to produce inactive mutants which were only cleaved at this inserted amino acid sequence, and thereby activated for cytolytic activity, by tumor proteases such as cathepsin B.

Based on these results, proimmunolysins were proposed [Panchal et al., Nature Biotechn. 14, 852 (1996)] which comprise an antibody which is coupled to a Staphylococcus aureus α-hemolysin which can be activated by tumor proteases or to a sea anemone equinatoxin II, with the antibody determining the target cell specificity of the coupling product.

While experimental results providing evidence in support of the antitumor activity of the antibody/toxin product were not presented, the proposed concept has, even without these data, to be regarded as being inadequate for a tumor therapy for the following reasons:

- The authors chose xenogenic nonendogenous lysins and/or toxins which are immunogenic for the host

organism (patient) and as result induce an immune reaction in the host organism, which immune reaction neutralize and inactivate the antibody/toxin conjugate.

- It is known [Sedlacek et al., Antibodies as Carriers of Cytotoxicity, Contrib. to Oncol. 43, Karger Verlag, Munich, 1992) that, due to their molecular size and to the rheological conditions at the tumor, tumor-specific antibodies and immunotoxins only accrue in very small quantity (0.01 - 0.001% of the given antibody or immunotoxin/g of tumor) at the tumor and only penetrate the tumor to an incomplete extent so that it is either not possible to destroy all the tumor cells or only possible to destroy a small portion of the tumor cells of a tumor.

- The extent to which tumor antigens, against which the antibody is directed, are expressed usually differs between the individual tumor cells, and variable, antigen-negative tumor cells evade the attack by the antibodies or the immunotoxins. Antigens which are secreted by the tumor cells neutralize the antibodies at the periphery of the tumor (review: Sedlacek et al., Monoclonal Antibodies in Tumor Therapy, Contrib. to Oncol., Karger Verlag, 1988).

In view of these inadequacies, there is still a great need for a target cell-specific therapy for tumors and inflammations. The present invention provides a novel technology which uses the secretion of enzymes in tumors or areas of inflammation to achieve the local release of active compounds whose inactive precursors (introduced by genes) are expressed in tumor cells, tumor-associated cells or inflammatory cells.

2) General description of the invention

In the simplest structure, the present invention relates to a nucleic acid construct which comprises the following components:

a) at least one promoter element
b) at least one DNA sequence for an active compound (protein B)
c) at least one DNA sequence for an amino acid sequence (part structure C) which can be cleaved specifically by an enzyme which is released by a mammalian cell
d) at least one DNA sequence for a peptide (part structure D) which, by means of binding by way of the amino acid sequence (part structure C) to the active compound (protein B), inhibits the activity of the active compound (protein B).

In their simplest form, the components can, for example, be arranged as depicted in Figure 1.

Expression of a protein BCD, encoded by components b), c) and d), is induced by activating the promoter sequence [component a)]. This protein BCD is cleaved in amino acid sequence C by cellular enzymes, e.g. proteases. This cleavage releases protein B, which constitutes the active compound. Within the meaning of the present invention, "proteases" (or "enzymes") means that one or more proteases (or enzymes) may be intended.

In conformity with the invention, the nucleotide sequence for component b) can be enlarged by a component b'). This component b') encodes a ligand (part structure B') which binds the active compound to a target structure.

Component b') can, for example, be arranged as depicted

in Figure 2.

Expression of the nucleic acid construct according to Figure 2) results in a protein B'BCD which binds to a target structure by way of the ligand (part structure B') and is cleaved by cellular proteases in part structure C, such that the active compound protein B'BD is released.

The novel nucleic acid constructs, or "gene constructs", are preferably composed of DNA. The term "nucleic acid constructs" is understood as meaning artificial structures which are composed of nucleic acid and which can be transcribed in the target cells. They are preferably inserted into a vector, with plasmid vectors or viral vectors being particularly preferred.

Depending on the choice of the promoter element [component a)], the novel nucleic acid constructs express a structural gene [components b) + c) + d) or b') + b) + c) + d)] either nonspecifically, cell-specifically, virus-specifically, under particular metabolic conditions, or cell-cycle specifically; alternatively, at least two different promoter elements are combined together to achieve an expression of the structural gene which is restricted in dependence on the choice of these promoter elements.

This structural gene encodes a protein which is cleaved in its part structure C by an enzyme. Enzymes are preferentially released in an organism by tumors and tumor cells and also by cells which are involved in an inflammatory process [review in Barrett et al., Mammalian Proteases, Academic Press, London 1980 and Sedlacek und Möröy, Immune Reactions, Springer Verlag, 1995)]. Examples of these enzymes are proteases, glycosidases and lipases.

This invention particularly relates to the choice of component c), such that the expressed protein B'BCD or BCD is preferentially cleaved in its part structure C by proteases which are formed in tumors or which are secreted by tumor cells or inflammatory cells. Examples of these proteases are plasminogen activators, such as plasminogen activator of the urokinase type or tissue plasminogen activator; cathepsins, such as cathepsin B, cathepsin D, cathepsin L, cathepsin E or cathepsin H, or their precursors (procathepsins); matrix metalloproteinases (MMP), such as collagenase of groups I, II, III, IV or V; stromelysin 1, stromelysin 2 or stromelysin 3; metrilysins; gelatinases, such as gelatinase A (MMP-2), and progelatinase B (MMP-9) and A [reviews in Pappot et al., Lung Cancer 12, 1 (1995), Schmitt et al., Fibronolysis 614, 3 (1992), Monsky et al., Cancer Biol. 4, 251 (1993), Rochefort et al., Medecine/Sciences 7, 30 (1991), Kao et al., 46, 1349 (1986), Fridman et al., Cancer Res. 55, 2548 (1995), Ray et al., Eur. Respir. J. 7, 2062 (1994), Cottam et al., Int. J. Oncol. 2, 861 (1993) and Tryggvason et al., Breast Cancer Res. and Treatm. 24, 209 (1993)]; tumor cell surface proteases [surface-expressed proteases = seprase; Monsky et al., Cancer Res. 54, 5702 (1994)]; elastase [Kao et al., Cancer Res. 46, 1355 (1986)]; prostate-specific antigen [Lundwall, Biochem. Biophys. Res. Commun. 161, 1151 (1989) and Riegman et al., Biochem. Biophys. Res. Commun. 159, 95 (1989)] or pancreatic trypsinogens [Miszuk-Jamska et al., FEBS Lett. 294, 175 (1991)]

Part structure B of the protein which is encoded by the novel structural gene constitutes the actual novel active compound which is released or activated by cleavage of part structure C and thereby converted from the inhibited state (protein BCD) into the active state (protein B).

In conformity with the invention, this active compound can

- be an enzyme which activates a biological activation cascade and/or is an active component of this cascade. Examples of such biological activation cascades are the coagulation system, the complement system or the kinin system
- be an enzyme which converts the inactive precursor of a pharmacological substance into the active substance
- or be a pharmacologically active substance.

Part structure B' of the protein which is encoded by the novel structural gene constitutes the novel ligand for binding the active compound (protein B) to a target structure.

A preferred target structure is the surface of cells, for example of endothelial cells, tumor cells, smooth muscle cells, fibroblasts, macrophages, lymphocytes, liver cells, kidney cells or cells of other tissues and organs.

A particularly preferred target structure is the surface of activated and/or proliferating endothelial cells.

Another preferred target structure is constituted by components of the extracellular matrix, such as collagens [reviews in Prockop et al., Annu. Rev. Biochem. 64, 403 (1995) and Wetzels et al., Am. J. Pathol. 139, 451 (1991)]; ficolin [Ichijo et al., J. Biol. Chem. 268, 14505 (1993)] sialoprotein [Bellahcene et al., Cancer Res. 54, 2823 (1994)]; laminin [von der Mark et al., Biochem. Biophys. Acta 823, 147 (1985) and Hunt, Expl. Cell Biol. 57, 165 (1989)]; proteoglycans [Schmidtchen et al., Biomed. Chromatography 7, 48 (1993)] or tenascin [Oyama et al., Cancer Res. 51, 4876

(1991) and Herlyn et al., Cancer Res. 51, 4853 (1991)].

The novel ligand (part structure B') can be

- an antibody or an antibody fragment such as Fab, Fv, single chain Fv or Fc, or
- a peptide or protein which binds to a receptor on the relevant cell membrane. Examples include cytokines, growth factors, their receptor-binding part sequences or peptide hormones, or
- an adhesion molecule or its adhesion sequence which bind to a corresponding molecule on the cell membrane or on the extracellular matrix, or
- the target cell-binding part, or the entire target cell-binding glycoprotein of a virus, or
- a peptide by means of which the active compound is anchored in the cell membrane of the cell which expresses it. Examples of these anchoring peptides are the transmembrane domains of receptors or virus proteins or else glycophospholipid anchors.

Component d) encodes a peptide (part structure D) which, bound to protein B or protein B'B by way of part structure C, inhibits the activity of protein B.

Component d) can be any arbitrary nucleic acid sequence. Preference is, however, given to nucleic acid sequences which encode endogenous peptides or proteins in order to avoid or reduce the risk of an immune reaction. In another preferred embodiment, components b) and d) contained in the novel structural gene encode endogenous proteins or peptides.

A considerable number of protein active compounds occur in nature in the form of inactive precursors (protein BClD). Such a precursor is activated by enzymes cleaving this precursor into a part structure which constitutes the actual protein active compound (protein B) and into

an inactive part structure (part structure D). This precursor is cleaved at at least one defined amino acid sequence, the so-called cleavage sequence (part structure C1).

This invention particularly relates to replacing this cleavage sequence (part structure C1), which occurs naturally in precursors of protein active compounds, with the part structure C. This replacement is effected by replacing the sequence encoding the part structure C1 in the nucleic acid sequence which encodes the natural precursor (protein BC1D) with component c) (encoding part structure C). After components a) and, where appropriate, b') have been added on, a novel gene construct is formed which is composed of components a) b') b) c) d) or a) b) c) d) and whose expression product protein B'BCD or BCD, respectively, is cleaved in part structure C by proteases which are formed in tumors or secreted by tumor cells or inflammatory cells, resulting in the formation of the active compound protein B'B or B.

The gene construct according to the invention, comprising components a) b) c) d) or a) b') b) c) d), is novel and does not occur in this form in nature.

Having been inserted into a nonviral or viral vector, the novel gene construct is administered locally or injected locally or into the circulation for the prophylaxis and/or therapy of diseases. These diseases particularly include tumor diseases and inflammations. Such inflammations can, for example, be elicited by physicochemical damage, by an infection or by an immune reaction against endogeneous or foreign tissue.

3) Detailed description of the components of the novel gene construct

The choice of the components of the novel gene construct

depends on the disease which is to be treated by administering the gene construct.

3.1) Promoter sequences [component a)]

Within the meaning of the invention, the following promoter sequences [component a)] are to be employed in the novel gene construct:

a) promoters and activator sequences which can be activated in an unrestricted manner, such as
   - the RNA polymerase III promoter
   - the RNA polymerase II promoter
   - the CMV promoter and enhancer
   - the SV40 promoter

b) viral promoter and activator sequences, such as
   - HBV
   - HCV
   - HSV
   - HPV
   - EBV
   - HTLV
   - HIV

When the HIV promoter is used, the entire LTR sequence including the TAR sequence [position $\leq$ -453 to $\geq$ -80, Rosen et al., Cell 41, 813 (1985)] is to be employed as a virus-specific promoter.

c) Metabolically activatable promoter and enhancer sequences, such as the hypoxia-inducible enhancer

d) Cell cycle-specifically activatable promoters, such as the promoter of the cdc25C gene, of the cyclin A gene, of the cdc2 gene, of the B-myb gene, of the DHFR gene or of the E2F-1 gene.

e) Tetracycline-activatable promoters, such as the tetracycline operator in combination with an appropriate repressor.

Within the meaning of the invention, use is to be made, as promoter sequences, of nucleotide sequences which, after binding transcription factors, activate the transcription of a structural gene which adjoins the 3' end.

f) Cell-specifically activatable promoters
These preferably include promoters, or activator sequences composed of promoters or enhancers, from those genes which encode proteins preferentially in selected cells.

For example, within the meaning of the invention, promoters for the following proteins are preferably to be used in the following cells:

f.1) Promoter and activator sequences which are activated in endothelial cells
- brain-specific, endothelial glucose-1 transporter
- endoglin
- VEGF receptor 1 (flt-1)
- VEGF receptor 2 (flk-1, KDR)
- til-1 or til-2
- B61 receptor (Eck receptor)
- B61
- endothelin, especially
- endothelin B
- endothelin 1
- endothelin receptors, in particular the endothelin B receptor
- mannose 6-phosphate receptors
- von Willebrand factor
- Il-1a, Il-1β
- Il-1 receptor

- vascular cell adhesion molecule (VCAM-1)
- synthetic activator sequences

As an alternative to natural, endothelial cell-specific promoters, it is also possible to use synthetic activator sequences which are composed of oligomerized binding sites for transcription factors which are preferentially or selectively active in endothelial cells. An example of this is the transcription factor GATA-2, whose binding site in the endothelin 1 gene is 5'-TTATCT-3' [Lee et al., Biol. Chem. 266, 16188 (1991), Dormann et al., J. Biol. Chem. 267, 1279 (1992) and Wilson et al., Mol. Cell. Biol. 10,. 4854 (1990)].

f.2) Promoters or activator sequences which are activated in cells in the vicinity of activated endothelial cells, in particular in smooth muscle cells

- VEGF

The regulatory sequences for the VEGF gene are

- the 5'-flanking region
- the 3'-flanking region
- the c-Src gene
- the v-Scr gene
- steroid hormone receptors and their promoter elements, in particular the mouse mammary tumor virus promoter
- tropomyosin
- α-actin
- α-myosin
- receptor for PDGF
- receptor for FGF
- MRF-4
- phosphofructokinase A
- phosphoglycerate mutase
- troponin C
- myogenen

- receptors for endothelin A
- desmin
- "artificial" promoters

Factors of the helix-loop-helix (HLH) family (myoD, myf-5, myogenen and MRF4 [review in Olson and Klein, Genes Dev. 8, 1 (1994)] are described as being muscle-specific transcription factors. The muscle-specific transcription factors also include the zinc finger protein GATA-4 [Arceci et al., Mol. Cell Biol. 13, 2235 (1993) and Ip et al., Mol. Cell Biol. 14, 7517 (1994)] and the MEF transcription factor groups [Yu et al., Gene Dev. 6, 1783 (1992)].

The HLH proteins and GATA-4 exhibit muscle-specific transcription not only with promoters of muscle-specific genes but also in a heterologous context, that is with artificial promoters as well. Examples of such artificial promoters are:

- multiple copies of the (DNA) binding site for muscle-specific HLH proteins, such as the E box (Myo D)
  (e.g. 4x AGCAGGTGTTGGGAGGC)
  [Weintraub et al., PNAS 87, 5623 (1990)]
- multiple copies of the DNA binding site for GATA-4 of the α-myosin heavy chain gene (e.g. 5'-GGCCGATGGGCAGATAGAGGGGGCCGATGGGCAGATAGAGG3')
  [Molkentin et al., Mol. Cell Biol. 14, 4947 (1994)].

f.3) Promoters and activator sequences which are activated in leukemia cells

Examples of these are promoters for

- c-myc
- HSP-70
- bcl-1/cyclin D-1
- bcl-2

- IL-6
- IL-10
- TNFα, TNFβ
- HOX-11
- BCR-Abl
- E2A-PBX-1
- PML-RATA

f.4) Promoters or activator sequences which are activated in tumor cells

A gene-regulatory nucleotide sequence with which transcription factors which are formed or are active in tumor cells interact is envisaged as the promoter or activator sequence.

Within the meaning of this invention, the preferred promoters or activator sequences include gene-regulatory sequences or elements from genes which encode proteins which are formed, in particular, in cancer cells or sarcoma cells. Thus, the promoter of the N-CAM protein is, for example, used in the case of small-cell bronchial carcinomas, while the promoter of the hepatitis growth factor receptor or of L-plastin is used in the case of ovarian carcinomas, and the promoter of L-plastin or of polymorphic epithelial mucin (PEM) is used in the case of pancreatic carcinomas.

f.5) Promoters and activator sequences which are activated in glia cells

These include, in particular, the gene-regulatory sequences or elements from genes which encode, for example, the following proteins:

- the Schwann cell-specific protein periaxin
- glutamine synthetase
- the glia cell-specific protein (glial fibrillary acid protein = GFAP)

- the gliacell protein S100b
- IL-6 (CNTF)
- 5-HT receptors
- TNFα
- IL-10
- insulin-like growth factor receptor I and II
- VEGF

The gene-regulatory sequences for the VEGF gene have already been listed above.

f.6) Promoters and activator sequences which are activated in lymphocytes and/or macrophages

These include, for example, the promoter and activator sequences of the genes for cytokines, cytokine receptors and adhesion molecules and receptors for the Fc fragment of antibodies.

Examples of these are:

- IL-1 receptor
- IL-1α
- IL-1β
- IL-2
- IL-2 receptor
- IL-3
- IL-3 receptor (α subunit)
- IL-3 receptor (β subunit)
- IL-4
- IL-4 receptor
- IL-5
- IL-6
- IL-6 receptor
- interferon regulatory factor I (IRF-1)
  (The promoter of IRF-1 is activated by IL-6 as well as by IFNγ or IFNβ.
- IFNγ-responsive promoter
- IL-7
- IL-8

- IL-10
- IL-11
- IFNγ
- GM-CSF
- GM-CSF receptor (α chain)
- IL-13
- LIF
- macrophage colony-stimulating factor (M-CSF) receptor
- Type I and II macrophage scavenger receptors
- MAC-1 (leukocyte function antigen)
- LFA-1α (leukocyte function antigen)
- p150,95 (leukocyte function antigen)

f.7) Promoter and activator sequences which are activated in synovial cells

These include the promoter sequences for matrix metalloproteinases (MMP), for example for:

- MMP-1 (interstitial collagenase)
- MMP-3 (stromelysin/transin)

They also include the promoter sequences for tissue inhibitors of metalloproteinases (TIMP), for example

- TIMP-1
- TIMP-2
- TIMP-3

f.8) Within the meaning of the invention, several of the promoter sequences which have been cited by way of example can be combined with each other in order to achieve the greatest possible target cell specificity in the expression of the novel gene construct. Two identical promoters can also be combined.

Several promoter sequences can, for example, be combined by way of:

- chimeric promoters
  A chimeric promoter is the combination of an upstream activator sequence, which can be activated cell-specifically, metabolically or virus-specifically, with a downstream promoter module which can bind the transcription factors of the CDF and CHF or E2F and CHF families and thereby inhibit activation of the upstream activator sequence in the G0 and G1 phases of the cell cycle [Lucibello et al., EMBO J. 14, 132 (1994), PCT/GB95/02000)].

- Hybrid promoters, for example in the form in which the TATA box of a promoter is mutated, with this mutation being compensated for by a corresponding mutation in the gene of a TATA-binding protein and this TATA-binding protein being under the control of another promoter.

3.2) Nucleic acid sequence [component b')] encoding a ligand (part structure B')

Within the context of the present invention, the ligand is a substance which binds a membrane antigen to a receptor or to an adhesion molecule on the target cell or is integrated into the cell membrane and/or binds to the extracellular matrix. [Review of the important cytokines and growth factors and their receptors, adhesion molecules and extracellular matrix proteins in von Ayad et al., The Extracellular Matrix, Academic Press 1994; Callard et al., The Cytokine, Academic Press 1994; Pigott et al., The Adhesion Molecule, Academic Press 1994 and Barclay et al., The Leucocyte Antigen, Academic Press 1994].

Examples of substances which bind to receptors are:

- growth factors such as VEGF, PDGF, EGF, TGFα, TGFβ,

KGF, SDGF, FGF, IGF, HGF, NGF, BDNF, neurotrophins, BMF, bombesin, M-CSF, thrombopoietin, erythropoietin, SCF, SDGF, oncostatin, PDEGF and endothelin-1

- cytokines, such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14 and IL-15
- interferon α, β and γ
- tumor necrosis factors TNFα and TNFβ
- chemokines, such as RANTES, MCAF, MIP-1α or -β, NAP and β-thromboglobulin
- peptide hormones, such as SRH, SIH or STH, MRH or MSH, PRH, PIH or prolactin, LH-RH, FSH-RH, LH/ICSH or FSH, TRH or TSH or CRH or ACTH
- angiotensin, kinins or homologs or analogs thereof
- vitamins, such as folic acid.

Within the context of the present invention, the ligand can also be an adhesion molecule, a part of the adhesion molecule or an analog of an adhesion molecule which binds to a corresponding cell membrane-located adhesion molecule or to another specific binding structure for an adhesion molecule on the target cell or the extracellular matrix.

Examples of such adhesion molecules which are able to function as ligands are

- Lewis X (for GMP-140)
- S-Lewis X (for ELAM-1)
- LFA-1 (for ICAM-1 and ICAM-2)
- MAC-1 (for ICAM-1)
- VLA-4 (for VCAM-1)
- PECAM (for PECAM)
- vitronectin (for the vitronectin receptor)
- GMP-140 (for Lewis X)
- S-Lewis X (for ELAM-1)
- ICAM-1, ICAM-2 (for LFA-1, MAC-1)

- VCAM-1 (for VLA-4)
- fibronectin (for VLA-4)
- laminin (for VLA-6)
- fibronectin, laminin (for VLA-1, VLA-2, VLA-3)
- fibronectin (for VLA-4)
- fibrinogen (for GPIIb-IIIa)
- B7 (for CD28)
- CD28 (for B7)
- CD40 (for CD40L)
- CD40L (for CD40)

Within the context of the present invention, the ligand can also be the extracellular part of an Fc receptor [Dougherty et al., Transfusion Science 17, 121 (1996)].

Furthermore, within the context of the present invention, the ligand can also be an antibody molecule or the epitope-binding part of an antibody molecule.

The murine monoclonal antibodies are preferably to be employed in humanized form. The humanization is effected in the manner described by Winter et al. [Nature 349, 293 (1991) and Hoogenbooms et al. (Rev. Tr. Transfus. Hemobiol. 36, 19 1993)].

Recombinant antibody fragments are either prepared directly from existing hybridomas or are isolated by means of phage display technology [Smith, Science 228, 1315 (1985)] from libraries of murine or human antibody fragments [Winter et al., Annu. Rev. Immunol. 12, 433 (1994)]. These antibody fragments are then employed directly, at the gene level, for further manipulations (e.g. fusion with other proteins).

In order to prepare recombinant antibody fragments from hybridomas, the genetic information which encodes the antigen-binding domains (VH, VL) of the antibodies is obtained by isolating the mRNA, reverse-transcribing the

RNA into cDNA and then amplifying the cDNA using the polymerase chain reaction [Saiki et al., Science 230, 1350 (1985)] and oligonucleotides which are complementary to the 5' and 3' ends, respectively, of the variable fragments (Orlandi et al., 1989). The VH and VL fragments are then cloned into bacterial expression vectors, e.g. in the form of Fv fragments [Skerra & Plückthun, Science 240, 1038 (1988)], single-chain Fv fragments (scFv) [Bird et al., Science 242, 423 (1988)] and Huston et al., PNAS-USA 85, 5879 (1988)] or as Fab fragments [Better et al., Science 240, 1041 (1988)].

Phage-display technology can also be used to isolate new antibody fragments directly from antibody libraries (immune libraries or naive libraries) of murine or human origin. In the phage display of antibody fragments, the antigen-binding domains are cloned, as fusion proteins together with the g3P coat protein of filamentous bacteriophages, either into the phage genome [McCafferty et al., Nature 348, 552 (1990)] or into phagemid vectors [Breitling et al., Gene 104, 147 (1991)] in the form of scFv fragments [MacCafferty et al., Nature 348, 552 (1990)] or as Fab fragments [Hoogenboom et al., Nucl. Acid Res. 19, 4133 (1991) and Barbas et al., PNAS-USA 88, 7978 (1991)]. Antigen-binding phages are selected on antigen-loaded plastic vessels (panning) [Marks et al., J. Mol. Biol. 222, 581 (1991)], on antigen-conjugated, paramagnetic beads [Hawkins et al., J. Mol. Biol. 226, 889 (1992)] or by binding to cell surfaces [Marks et al., Bio/Technol. 11, 1145 (1993)].

Immune libraries are prepared by subjecting the variable antibody fragments from B lymphocytes of immunized animals [Sastry et al., PNAS-USA 86, 5728 (1989), Ward et al., Nature 341, 544 (1989), Clackson et al., Nature 352, 624 (1991)] or patients [Mullinax et al., PNAS-USA 87, 8095 (1990), Barbas et al., PNAS-USA 88, 7978

(1991)] to PCR amplification. For this, use is made of combinations of oligonucleotides which are specific for murine [Orlandi et al., PNAS-USA 86, 3833 (1989), Sastry et al., PNAS-USA 86, 5728 (1989)] or human immunoglobulin genes [Larrick et al., BBRC 160, 1250 (1989)] or for the human immunoglobulin gene families [Marks et al. Eur. J. Immunol. 21, 985 (1991)].

Naive libraries can be prepared using nonimmunized donors as the source of the immunoglobulin genes [Marks et al., J. Mol. Biol. 222, 581 (1991)]. Alternatively, immunoglobulin germ line genes can be employed to prepare semisynthetic antibody repertoires, with the complementarity-determining region 3 of the variable fragments being amplified by PCR using degenerate primers [Hoogenboom & Winter, J. Mol. Biol. 227, 381 (1992), Barbas et al., PNAS-USA 89, 4457 (1992), Nissim et al., EMBO J. 13, 692 (1994) and Griffiths et al., EMBO J. 13, 3245 (1994)]. As compared with immune libraries, these so-called single pot libraries have the advantage that antibody fragments against a large number of antigens can be isolated from one single library [Nissim et al., EMBO J. 13, 692 (1994)].

Phage-display technology can be used to increase the affinity of antibody fragments still further, with new libraries being prepared from already existing antibody fragments by random [Hawkins et al., J. Mol. Biol. 226, 889 (1992), Gram et al., PNAS-USA 89, 3576 (1992)], codon-based [Glaser et al., J. Immunol. 149, 3903 (1992)] or site-directed mutagenesis [Balint & Larrick, Gene 137, 109 (1993)], by shuffling the chains of individual domains with those of fragments from naive repertoires [Marks et al., Bio/Technol. 10, 779 (1992)] or by using bacterial mutator strains [Low et al., J. Mol. Biol. 260, 359 (1996)], and antibody fragments having improved properties being isolated by reselecting under stringent conditions [Hawkins et al., J. Mol.

Biol. 226, 889 (1992)]. In addition, murine antibody fragments can be humanized by stepwise replacement of one of the variable domains with a human repertoire and then selecting with the original antigen (guided selection) [Jespers et al., Bio/Technol. 12, 889 81994)]. Alternatively, murine antibodies are humanized by specifically replacing the hypervariable regions of human antibodies with the corresponding regions of the original murine antibody [Jones et al., Nature 321, 522 (1987)].

Within the context of the present invention, the ligand can also be the nucleotide sequence encoding a coat protein, or a part of the coat protein, of viruses which bind specifically to selected cells by way of their coat protein.

The ligand can also be a peptide with whose help the active compound (protein B) is anchored in the cell membrane of the in expressing cells.

These anchoring peptides include

- Transmembrane domains of cell membrane-located receptors or of virus proteins, such as the transmembrane sequence of human macrophage colony-stimulating factor [DNA position $\leq$ 1485 to $\geq$ 1554; Cosman et al., Behring Inst. Mitt. 83, 15 (1988)] or the DNA sequence for the signal and transmembrane region of human respiratory syncytial virus (RSV) glycoprotein G [amino acids 1 to 63 or their part sequences, amino acids 38 to 63; Vijaya et al., Mol. Cell Biol. 8, 1709 (1988) and Lichtenstein et al., J. Gen. Virol. 77, 109 (1996)] or the DNA sequence for the signal and transmembrane region of influenza virus neuraminidase [amino acids 7 to 35 or the part sequence amino acids 7 to 27, Brown et al.,

J. Virol. 62, 3824 (1988)].

- However, the nucleotide sequence for a glycophospholipid anchor [review of glycophospholipid-anchored membrane proteins in Ferguson et al. (Ann. Rev. Biochem. 57, 285 (1988)] can also be inserted in order to anchor the active compound in the cell membrane of the transduced cells which are forming the active compound.

  Glycophospholipid anchors have been described, for example, for CEA [DNA position < 893 to > 1079; Berling et al., Cancer Res. 50, 6534 (1990)], for N-CAM [Cunningham et al., Science 236, 799 (1987)] and for other membrane proteins such as Thy-1 [Clissold, Biochem. J. 281, 129 (1992)] or CD16 [Selvaray et al., Nature 333, 565 (1988)].

The choice of the ligand depends on the target cell which is to be transduced with the gene construct.

Examples are:

a) Ligands for activated endothelial cells

Within the meaning of the invention, these include antibodies or antibody fragments which are directed against membrane structures of endothelial cells, as have been described, for example, by Burrows et al. [Pharmac. Ther. 64, 155 (1994), Hughes et al. (Cancer Res. 49, 6214 (1989) and Maruyama et al. (PNAS-USA 87, 5744 (1990)]. In particular, these antibodies include antibodies against actin or angiotensin II receptors, antibodies against receptors for growth factors such as VEGF, FGF, PDGF or EGF, and antibodies against adhesion molecules such as against the vitronectin receptor or ICAM-3.

The ligands furthermore include all active compounds which bind to membrane structures or membrane receptors on endothelial cells. Examples of these are IL-1 or growth factors or their fragments, or part sequences thereof, which bind to receptors which are expressed by endothelial cells, for example PDGF, bFGF, VEGF or TGFβ [Pusztain et al., J. Pathol. 169, 191 (1993)].

They furthermore include adhesion molecules which bind to activated and/or proliferating endothelial cells. Adhesion molecules of this nature, such as Slex, LFA-1, MAC-1, LECAM-1, VLA-4 or vitronectin, have already been described [reviews in Augustin-Voss et al., J. Cell Biol. 119, 483 (1992), Pauli et al., Cancer Metast. Rev. 9, 175 (1990), Honn et al., Cancer Metast. Rev. 11, 353 (1992) and Pigott et al., The Adhesion Molecule, Academic Press (1994)].

Within the meaning of this invention, the ligands particularly include the coat glycoproteins of viruses which have a tropism for endothelial cells. Examples of these viruses are:

- Filoviruses, for example
  - Marburg virus
    with its coat protein GP (glycoprotein) and sGP (second glycoprotein)
  - or Ebola virus, in each case with its coat protein GP and sG
- cytomegalovirus, in particular with its gB protein
- type I herpes simplex virus
- HIV-1 virus
- measles virus
- Hantaan virus
- alpha viruses, such as Semliki Forest virus
- epidemic, hemorrhagic fever virus
- polio virus
- enteroviruses (such as Echo 9, Echo 12 and

Cochsackie B3)

b) Ligands for muscle cells

Examples of these are antibodies or antibody fragments which are directed against membrane structures of muscle cells, in particular of smooth muscle cells. Examples of such antibodies are

- antibody 10F3, or
- antibodies against actin, or
- antibodies against angiotensin II receptors, or
- antibodies against receptors for growth factors, or
- antibodies which are directed, for example, against
  - EGF receptors
  - or against PDGF receptors
  - or against FGF receptors
  - or antibodies against endothelin A receptors.

The ligands furthermore include nucleotide sequences for active substances which bind to membrane structures or membrane receptors on muscle cells [review in Pusztai et al., J. Pathol. 169, 191 (1993) and Harris, Curr. Opin. Biotechnol. 2, 260 (1991)]. For example, they include growth factors or their fragments, or part sequences thereof, which bind to receptors which are expressed by smooth muscle cells, for example

- PDGF
- EGF
- TGFβ
- TGFα
- FGF
- endothelin A

However, within the meaning of this invention, the ligands also include coat glycoproteins of those viruses which have a tropism for muscle cells. Cytomegalovirus [Speir et al., Science 265, 391 (1994)] is an example of

these viruses.

c) Ligands for activated macrophages and/or activated lymphocytes

Within the meaning of the invention, the ligands also include nucleotide sequences for substances which bind specifically to the surface of immune cells. These substances include antibodies or antibody fragments which are directed against membrane structures of immune cells, as have been described, for example, by Powelson et al., Biotech. Adv. 11, 725 (1993) and Barclay et al., The Leucocyte Antigen, Academic Press (1994).

The ligands also include monoclonal or polyclonal antibodies or antibody fragments which bind, by their antigen-binding variable part, to Fc-γ or Fc-ε or Fc-μ receptors of immune cells [Rojanasakul et al., Pharm. Res. 11, 1731 (1994)].

They also include the Fc fragment of human monoclonal or polyclonal immunoglobulin.

The ligands furthermore include all substances which bind to membrane receptors on the surface of immune cells. These substances include cytokines, such as IL-1, IL-2, IL-3, IL-4, IL-6, IL-10, TNFα, GM-CSF and M-CSF, and also growth factors, such as EGF, TGF, FGF, IGF or PDGF or their fragments, or part sequences thereof, which bind to receptors which are expressed by immune cells [Callard et al., The Cytokine, Academic Press (1994)].

They furthermore include adhesion molecules and other ligands which bind to cell membrane structures, on macrophages in spleen, liver, lung and other tissue [Pigott et al., The Adhesion Molecule, Academic Press (1994) and Perales et al., Eur. J. Biochem. 226, 255

(1994)].

However, within the meaning of this invention, the ligands also include coat glycoproteins of those viruses which have a tropism for lymphocytes and/or macrophages.

Examples of these macrophage-infecting viruses are:

- HIV-1
  in particular those strains which have mutations in the V3 region of gp120 which lead to an increased binding to macrophages
- HIV-2
- Hantaviruses, for example Punmalavirus
- cytomegalovirus
- respiratory syncytial virus
- herpes simplex virus
- filoviruses

Examples of lymphocyte-infecting viruses are:

- varicella zoster virus (VZV);
  VZV infects T cells in particular
  herpes virus 6 (HHV-6);
  HHV-6 infects T cells in particular
- rabies virus;
  the rabies virus coat protein binds to TH2 cells in particular
- HIV-1;
  glycoprotein gp120 binds preferably to the CD4 molecule of T cells
- HTLV-II;
  HTLV-II infects B cells in particular
- HTLV-I;
  HTLV-I infects T cells in particular
- influenza C viruses;
  influenza C viruses bind, by way of the hemagglutinin esterase fusion (HEF) protein, to N-

acetyl-9-β-acetylneuraminic acid (Neu 5,9 Ac), which occurs preferentially on B lymphocytes and to a lesser extent, or not at all, on T lymphocytes

- influenza C viruses which have a mutation in nucleotide position 872 (which encodes position 284 of the HEF of the amino acid sequence), for example a replacement of threonine with isoleucine. The surface protein HEF which possesses this mutation has a markedly stronger affinity for the N-acetyl-9-0-acetylneuraminic acid receptor than does the wild-type virus
- influenza C virus HEF cleavage products which contain the structure for binding N-acetyl-9-β-acetyl-neuraminic acid. This binding structure is defined by the catalytic triad serine 71, histidine 368 or 369 and aspartic acid 261
- Epstein-Barr virus
  EBV infects B cells in particular
- herpes simplex virus 2;
  HSV-2 infects T cells in particular
- measles virus

d) Ligands for synovial cells and inflammatory cells

These ligands include nucleic acid sequences for antibodies or antibody fragments which bind, by their variable domains, to membrane structures of synovial cells or inflammatory cells. Examples of these membrane structures are

- vimentin [Miettinen et al., Am. J. Pathol. 117, 18 (1984)]
- fibronectin [Wojciak et al., Clin. Exp. Immunol. 93, 108 (1993)] or Fc receptors.

They also include antibodies or antibody fragments which bind, by their constant domains, to Fc receptors [Rojanasakul et al., Pharm. Res. 11, 1731 (1994)].

They furthermore include all active compounds which bind to membrane structures or membrane receptors on synovial cells. Examples of these active compounds are cytokines or growth factors or their fragments, or part sequences thereof, which bind to receptors which are expressed by synovial cells, for example IL-1-RA, TNFα, IL-4, IL-6, IL-10, IGF and TGFβ [Callard et al., The Cytokine, Academic Press (1994)].

e) Ligands for virus-infected cells

The ligands include nucleic acid constructs for antibodies or antibody fragments which are directed against the virus antigens which are expressed on the cell membrane of virus-infected cells.

Antibodies of this nature are directed, for example, against antigens of the following viruses:

- HBV
- HCV
- HSV
- HPV
- HIV
- EBV
- HTLV

f) Ligands for liver cells and other tissue cells

The ligands include all substances which bind to membrane structures or membrane receptors on the surface of liver cells. Examples of these ligands are growth factors, such as cytokines, EGF, TGF, FGF or PDGF, or their fragments or part sequences thereof, which bind to receptors which are expressed by cells of this nature.

They furthermore include ligands which bind to cell

membrane structures which are selective for particular tissues. Examples are:

Membrane structure	Ligand	Tissue cells
Transferrin receptor	Transferrin	Liver, other tissue cells
Insulin receptor	Insulin	Liver, other tissue cells
Fc-γ receptors	Immunoglobulin G	Reticuloendothelial system, other tissues

These ligands and membrane structures are reviewed in Perales et al., Eur. J. Biochem. 226, 255 (1994).

However, within the meaning of the invention, the ligands particularly include coat glycoproteins of viruses which have a tropism for selected cells, for example for

g) bronchial epithelial cells
   - respiratory syncytial virus

h) liver cells
   - hepatitis C virus
   - filoviruses
     liver cells bind Marburg virus, for example, by way of the asialoglycoprotein receptor
   - hepatitis B virus
     liver cells preferentially bind to the preS2 and preS1 domain of HBV by way of the asialoglycoprotein receptor
   - hepatitis D virus
   - liver sinusoidal cells
   - hepatitis V virus
     HBV is bound by way of fibronectin

i) Ligands for glia cells

These include nucleic acid sequences which encode anti-

bodies or antibody fragments which are directed against membrane structures of glia cells, as have been described, for example, by Mirsky et al. [Cell and Tissue Res. 240, 723 (1985)], Coakham et al. [Prog. Exp. Tumor Res. 29, 57 (1985)] and McKeever et al. [Neurobiol. 6, 119 (1991)]. These membrane structures furthermore include neural adhesion molecules such as N-CAM, in particular its polypeptide chain C [Nybroe et al., J. Cell Biol. 101, 2310 (1985)].

They furthermore include all active compounds which bind to membrane structures or membrane receptors on glia cells. Examples of these active compounds are insulin and insulin-like growth factor and those fragments of these growth factors which bind to the appurtenant membrane receptors.

Within the meaning of the invention, the ligands also include nucleic acid sequences which encode coat glycoproteins of those viruses which have a tropism for glia cells.

Examples of these viruses are:

- HIV-1, subtype JRF1
- herpes simplex virus I

k) Ligands for leukemia cells

These include nucleic acid constructs which encode antibodies or antibody fragments which are directed against membrane structures of leukemia cells. A large number of monoclonal antibodies of this nature have already been described for diagnostic and therapeutic methods [reviews in Kristensen, Danish Medical Bulletin 41, 52 (1994); Schranz, Therapia Hungarica 38, 3 (1990); Drexler et al., Leuk. Res. 10, 279 (1986); Naeim, Dis. Markers 7, 1 (1989); Stickney et al., Curr. Opin. Oncol.

4, 847 (1992); Drexler et al., Blut 57, 327 (1988) and Freedman et al., Cancer Invest. 9, 69 (1991)]. Depending on the type of leukemia, suitable ligands are, for example, monoclonal antibodies, or their antigen-binding antibody fragments, of the following specificity:

Cells	Membrane antigen
AML	CD13
	CD14
	CD15
	CD33
	CAMAL
	sialosyl-Le
B-CLL	CD5
	CD1c
	CD23
	idiotypes and isotypes of membrane immunoglobulins
T-CLL	CD33
	M38
	IL-2 receptors
	T cell receptors
ALL	CALLA
	CD19
	non-Hodgkin's lymphoma

The ligands furthermore include all active compounds which bind to membrane structures or membrane receptors of leukemia cells. Examples of these active compounds are growth factors, or their fragments or part sequences thereof, which bind to receptors which are expressed by leukemia cells.

Growth factors of this nature have already been described [reviews in Cross et al., Cell 64, 271 (1991); Aulitzky et al., Drugs 48, 667 (1994); Moore, Clin. Cancer Res. 1, 3 (1995) and Van Kooten et al., Leuk. Lymph 12, 27 (1993)]. Examples are:

- IFNα in the case of non-Hodgkin's lymphomas
- IL-2, particularly in the case of T cell leukemias
- FGF in the case of T cell, monocytic, myeloid, erythroid and megarkaryoblastic leukemias
- TGFβ in the case of leukemias
- retinoids, e.g. retinoic acid in the case of acute promyelocytic leukemia

1) Ligands for tumor cells

These include nucleic acid sequences encoding antibodies, and fragments of these antibodies, which are directed against membrane structures on tumor cells. Antibodies of this nature have been reviewed, for example, by Sedlacek et al., Contrib. to Oncol. 32, Karger Verlag, Munich (1992) and Contrib. to Oncol. 43, Karger Verlag, Munich (1992).

Other examples are antibodies against:

- sialyl Lewis
- peptides on tumors which are recognized by T cells
- proteins which are expressed by oncogenes
- gangliosides, such as GD3, GD2, GM2, 9-0-acetyl GD3 and fucosyl GM1
- blood group antigens and their precursors
- antigens on polymorphic epithelial mucin
- antigens on heat shock proteins

3.3) Nucleic acid sequence [component b)] encoding an active compound (protein B)

The active compound (protein B) according to the invention is a substance which, for example, intervenes in a biological activation cascade and/or is an active component of this cascade. These substances include active compounds which

a) activate the coagulation cascade, for example:
- thrombin [MacGillivray et al., Ann. N.Y. Acad. Sci. 485, 73 (1986)]
- thrombin which is mutated in the region of the Arg-Thr cleavage site (amino acid position 327/328)
- factor Va [Cripe et al., Biochem. 31, 3777 (1992) and Jenny et al., PNAS USA 84, 4846 (1987)]
- factor VIIa [O'Hara et al., PNAS USA 84, 5158 (1987)]
- factor IXa (Yoshitake et al., Biochem. 24, 3736 (1985)]
- factor Xa [Messier et al., Gene 99, 291 (1991)]
- tissue factor and fragments of tissue factor which are active in coagulation [Morrissey et al., Cell 50, 29 (1987); Scarpati et al., Biochem. 26, 5234 (1987); Spicer et al., PNAS USA 84, 5148 (1987) and Rehemtulla et al., Thromb. Heamost. 65, 521 (1991)]

b) inhibit the coagulation cascade or activate fibrinolysis,
for example
- the plasminogen activator inhibitors PAI-1, PAI-2 and PAI-3
- hirudin
- protein C
- serine proteinase inhibitors, such as
  - C-1S inhibitor
  - α1-antitrypsin
  - antithrombin III
- tissue factor pathway inhibitor (TFPI)
- plasminogen activators such as urokinase, tissue plasminogen activator (tPA), or hybrids thereof

c) activate the complement cascade, for example
- cobra venum factor (CVF) or part sequences of CVF which correspond functionally to human

complement factor C3b, i.e. which are able to bind to complement factor B and, after cleavage by factor D, constitute a C3 convertase. The DNA sequence for CVF and its part sequences were published by Fritzinger et al., Proc. Natl. Acad. Sci. USA 91, 12775 (1994).

- Human complement factor C3b. The DNA sequence for C3 and its part sequences has been published by De Bruijn et al., Proc. Natl. Acad. Sci. USA 82, 708 (1985).
- Cleavage products of human complement factor C3 which resemble CVF functionally and structurally. Cleavage products of this nature have been described by O'Keefe et al., J. Biol. Chem. 263, 12690 (1988).

d) Activate the kinin system, the complement system and/or the coagulation system, for example

- activated Hagemann factor (F XIIa) [Shibuya et al., Biochem. Biophys. Acta 1206, 63 (1994), Que et al., Biochem. 25, 1525 (1986) and Tripodi et al., Nucl. Acid Res. 14, 3146 (1986)]
- Kallikrein [Chen et al., Biochem. J. 307, 481 (1995), Fukushima et al., Biochem. 24, 8037 (1985)]

e) The active compound (protein B) according to the invention is furthermore a cytostatic, cytotoxic or inflammatory protein, for example:

- perforin
- granzyme
- cytokines, such as
  - IL-1
  - IL-2
  - IL-4
  - IL-12
  - IL-3
  - IL-5

- human leukemia inhibitory factor (LIF)
- IL-7
- IL-11
- IL-13
- GM-CSF
- G-CSF
- M-CSF

- interferons, such as
  - IFNα
  - IFNβ
  - IFNγ
- TNF
  - TNFα
  - TNFβ
- oncostatin M
- sphingomyelinase [Jarvis et al., PNAS USA 91, 73 (1994)]
- magainin and magainin derivatives [Cruciani et al., PNAS USA 88, 3792 (1991); Jacob et al., Ciba Found. Symp. 186, 197 (1994) and Peck-Miller et al., Cancer Chemother. Pharmac. 32, 109 (1993)]
- chemokines, such as
  - RANTES (MCP-2)
  - monocyte chemotactic and activating factor (MCAF)
  - IL-8
  - macrophage inflammatory protein-1 (MIP-1α, -β) neutrophil activating protein-2 (NAP-2)

f) The active compound (protein B) according to the invention is also an antiangiogenic protein, such as

- angiostatin
- interferons
  - IFNα
  - IFNβ
  - IFNγ

- platelet factor 4
- IL-12
- TIMP-1
- TIMP-2
- TIMP-3

g) The active compound (protein) according to the invention is additionally an enzyme which is able to convert an inactive precursor of a pharmacological active substance (e.g. a cytostatic agent) into the active substance.

Examples of these enzymes are:

- bacterial nitroreductase
- bacterial β-glucuronidase
- plant β-glucuronidase obtained from Secale cereale
- human β-glucuronidase
- human carboxypeptidase (CB), for example
  - mast cell CB-A
  - pancreatic CB-B
  - bacterial carboxypeptidase
- bacterial β-lactamase
- bacterial cytosine deaminase
- human catalase or peroxidase
- phosphatase, in particular
  - human alkaline phosphatase
  - human acid prostate phosphatase
  - type 5 acid phosphatase
- oxidase, in particular
  - human lysyloxidase
  - human acid D-aminooxidase
- peroxidase, in particular
  - human gluthatione peroxidase
  - human eosinophil peroxidase
  - human thyroid peroxidase

The active compounds (protein B) within the meaning of the invention is also a protein which affects the immune

system,

h) for example a protein having an antiallergic effect, such as
- IFNβ
- IFNγ
- IL-10
- soluble IL-4 receptors
- IL-12
- TGFβ

i) or a protein which can prevent the rejection of transplanted organs, such as
- IL-10
- TGFβ
- soluble IL-1 receptors
- soluble IL-2 receptors
- IL-2 receptor antagonists
- soluble IL-6 receptors

k) or a protein for the therapy of antibody-mediated autoimmune diseases, for example
- TGFβ
- IFNα
- IFNβ
- IFNγ
- IL-12
- soluble IL-4 receptors
- soluble IL-6 receptors

l) or a protein for the therapy of cell-mediated autoimmune diseases, for example
- IL-6
- IL-9
- IL-10
- IL-13
- TNFα
- IL-4
- TNFβ

m) or a protein for the therapy of arthritis

Within the meaning of the invention, structural genes are selected whose expressed protein directly or indirectly inhibits the inflammation, for example in a joint, and/or promotes the reconstitution of extracellular matrix (cartilage, connective tissue) in a joint.

Examples of these expressed proteins are

- IL-1 receptor antagonists (IL-1-RA);
  IL-1-RA inhibits the binding of IL-1α and IL-1β
- soluble IL-1 receptor;
  soluble IL-1 receptor binds and inactivates IL-1
- IL-6;
  IL-6 increases the secretion of TIMP and superoxides and decreases the secretion of IL-1 and TNFα by synovial cells and chondrocytes
- soluble TNF receptor;
  soluble TNF receptor binds and inactivates TNF
- IL-4;
  IL-4 inhibits the formation and secretion of IL-1, TNFα and MMP
- IL-10;
  IL-10 inhibits the formation and secretion of IL-1, TNFα and MMP and increases the secretion of TIMP
- insulin-like growth factor (IGF-1);
- IGF-1 stimulates the synthesis of extracellular matrix
- TGFβ
  especially TGFβ1 and TGFβ2
- TGFβ stimulates the synthesis of extracellular matrix superoxide dismutase
- TIMP (tissue inhibitors of metalloproteinases)
  especially
  - TIMP-1
  - TIMP-2

- TIMP-3

The active compound (protein B) within the meaning of the invention is furthermore a protein for repairing damage to the nervous system, for example

n) a growth factor, such as
- FGF
- nerve growth factor (NGF)
- brain-derived neurotrophic factor (BDNF)
- neurotrophin-3 (NT-3)
- neurotrophin-4 (NT-4)
- ciliary neurotrophic factor (CNTF)

o) or a cytokine or a cytokine inhibitor which is able to inhibit or neutralize the neurotoxic effect of TNFα, for example for
- TGFβ
- soluble TNF receptors
- IL-10;
  IL-10 inhibits the formation of IFNγ, TNFα, IL-2 and IL-4
- soluble IL-1 receptors
- IL-1 receptor I
- IL-1 receptor II;
  soluble IL-1 receptors neutralize the activity of IL-1
- IL-1 receptor antagonist
- soluble IL-6 receptors

p) The active compound (protein B) within the meaning of the invention is also a protein which stimulates angiogenesis, for example
- VEGF
- FGF

q) The active compound (protein B) within the meaning of the invention is also a protein which lowers

blood pressure, for example
- kallikrein
- endothelial cell nitric oxide synthase

r) The active compound (protein B) within the meaning of the invention is also a protein for the therapy of chronic infectious diseases, for example
- a protein which exhibits cytostatic or cytotoxic effects. (Examples of cytotoxic or cytostatic proteins have already been cited) or
- an enzyme which cleaves a precursor of an antiviral or cytotoxic substance into the active substance. (Examples of enzymes of this nature have already been cited) or
- a cytokine which has an antiviral effect or a growth factor which has an antiviral effect. Examples of these are
  - IFNα
  - IFNβ
  - IFNγ
  - TNFβ
  - TNFα
  - IL-1
  - TGFβ

3.4) Combination of identical or different structural genes

The invention furthermore relates to a gene construct in which a combination of the DNA sequences of two identical or two different DNA sequences for active compounds (protein B) [component b) and b")] is present. In order to express the two DNA sequences, the cDNA of an internal ribosome entry site (IRES) is preferably intercalated, as a regulatory element, between the two structures.

An internal ribosome entry site enables two DNA

sequences which are linked to each other by way of an IRES to be expressed.

IRESs of this nature have been described, for example, by Montford and Smith [TIG 11, 179 (1995); Kaufman et al., Nucl. Acids Res. 19, 4485 (1991); Morgan et al., Nucl. Acids Res. 20, 1293 (1992); Dirks et al., Gene 128, 247 (1993); Pelletier and Sonenberg, Nature 334, 320 (1988) and Sugitomo et al., BioTechn. 12, 694 (1994)].

Thus, the cDNA of the polio virus IRES sequence (position $\leq$ 140 to $\geq$ 630 of the 5' UTR [Pelletier and Sonenberg, Nature 334, 320 (1988)] can be used to link the DNA of component c) to the DNA of component d).

3.5) Nucleic acid sequences [component c)] for the protease-cleavable part structure C

According to the invention, part structure C contains an amino acid sequence which is cleaved by proteases which are formed in tumors or by tumor cells or by inflammatory cells. The nucleic acid sequence for this part structure C is, for example, inserted into the nucleic acid sequence of the naturally occurring precursor (protein BC1D, where C1 constitutes the naturally occurring cleavage sequence) of the respective active compound (protein B) in place of the cleavage sequence C1 such that the protein BCD or B'BCD is expressed by this recombinant nucleic acid.

The protease which is predominantly secreted in the tumor or in the inflammation determines the choice of the nucleic acid sequence which encodes part structure C.

For example, the following part structures C can be employed for the following enzymes [Barrett et al.,

Mammalian Proteases, Academic Press, London (1980), Panchal et al., Nature Biotechnol. 14, 852 (1996); Pigott et al., Ayad et al., The extracellular Matrix, Academic Press (1994); Yoshida et al., Int. J. Cancer 63, 863 (1995), Petersen et al., J. Biol. Chem. 265, 6104 (1990); Cramer et al., J. Urology 156, 526 (1995); Forsgen et al., FEBS Lett. 213, 254 (1987) and Zhang et al. Chin. Chem. 41, 1567, 1995)]:

Enzyme	S4	S3	S2	S1	Part structure C Cleavage - S-1	(S-2)
Plasminogen activator	Cys	Pro	Gly	Arg	Val	(Val)
		Gln	Gly	Arg		
		Gly	Gly	Arg		
		Gly	Ly	Arg		
Prostate-specific antigen	Pro	Arg	Phe	Lys	Ile	(Ile)
		Arg	Pro	Tyr		
	Arg	Arg	Phe	Phe	Leu	(His)
Cathepsins	Pro	Arg	Phe	Lys	Ile	(Ile)
				Tyr		
		Lys	Ser	Arg	Met	
		Lys	Met	Arg	Arg	
		Ile	Arg	Arg	Arg	
		Arg	Ala	Arg	Leu	
		Gln	Ala	Arg	Phe	
		Lys	Leu	Arg	Leu	
			Lys	Arg	Val	
				Lys		
			Phe	Arg		
Stromelysins	Gly	Gly	Gly	Ala	Gln	(Leu)
	Gln	Leu	Gly	Val	Met	(Gln)
	Ala	Ala	Ala	Ser	Leu	(Lys)
	Val	Ala	Val	Ser	Ala	(Lys)
	Leu	Ala	Ala	Asn	Leu	(Arg)
Collagenase I	Gly	Pro	Gln	Gly	Ile	(Ala)
	Gly	Pro	Gln	Gly	Leu	(Leu)

II	Gly	Pro	Gln	Gly	Leu	(Ala)
III	Gly	Ile	Ala	Gly	Ile	(Thr)
VIII	Gly	Leu	Pro	Gly	Ile	(Gly)
	Gly	Phe	Pro	Gly	Ile	(Gly)
XI	Gly	Pro	Ala	Gly	Ile	(Ser)
	Gly	Pro	Ala	Gly	Ile	(Ala)

The aminopositions (S1-S4 and and S-1 and S-2) were defined in accordance with Schechter and Berger, Biochem. Biophys. Res. Comm. 27, 157 (1967).

3.6) Nucleic acid sequences [component d)] encoding part structure D

According to the invention, the nucleic acid sequence [component d)] encodes a peptide (part structure D) which binds, by way of part structure C, to the active compound (part structure B) and inactivates the latter by this binding.

Those nucleic acid sequences which encode part structure D in the natural precursors (protein BC1D), with part structure C1 constituting the natural cleavage sequence in protein BC1D, are preferably taken for part structure D.

The structures of the natural precursors of active compounds (protein B) have already been reviewed, thus, for example

for coagulation factors, complement factors and kallikrein
[Bartett et al., Mammalian Proteases, Academic Press, London (1980)]
for interleukins, chemokines and growth factors
[Callard et al., The Cytoline Facts Book, Academic Press (1994)]
for tissue inhibitor of metalloproteinases (TIMPs)

[Denhardt et al., Pharmac. Ther. 59, 329 (1993)].

When selecting active compounds which do not possess any naturally occurring precursors, and in the case of xenogenic active compounds, nucleic acid sequences encoding any arbitrary peptides are to be used as component d), preferably, however, nucleic acid sequences which encode those part structures D which occur naturally in the precursors of human active compounds.

3.7) Insertion of signal sequences

In order to facilitate secretion of the protein BCD or B'BCD which is expressed from the novel nucleic acid sequence, the homologous signal sequence which may possibly be present in the DNA sequence of component b) can be replaced by a heterologous signal sequence which improves extracellular secretion.

Thus, for example, the signal sequence for immunoglobulin [DNA position $\leq$ 63 to $\geq$ 107; Riechmann et al., Nature 332, 323 (1988)] or the signal sequence for CEA [DNA position $\leq$ 33 to $\geq$ 134, Schrewe et al., Mol. Cell Biol. 10, 2738 (1990) or Berling et al., Cancer Res. 50, 5634 (1990)] or the signal sequence of human respiratory syncytial virus glycoprotein [cDNA for amino acids $\leq$ 38 to $\geq$ 50 or 48 to 65; Lichtenstein et al., J. Gen. Virol. 77, 109 (1996)] can be inserted.

Furthermore, in order to augment translation, the nucleotide sequence GCCACC or GCCGCC [Kozak, J. Cell Biol. 108, 299 (1989)] can be inserted at the 3' end of the promoter sequence and directly at the 5' end of the start signal (ATG) of the signal sequence.

3.8) Application of the novel nucleic acid construct

The novel nucleic acid construct can be used for prepar-

ing drugs for treating diseases which are associated with an increased local formation of proteases, for example tumor diseases, leukemias, allergies, autoimmune diseases, infections, inflammations, transplant rejection reactions, thromboses and blood vessel aclusions and other blood coagulation and blood circulation disturbances, and tissue injuries, including injuries to the central nervous system. The drugs are administered locally (e.g. onto the skin, nasally, orally, gastrointestinally, intrabronchially, intravesicularly, intravaginally, into the uterus, subcutaneously, intramuscularly, periarticularly, intraarticularly, into the cerebrospinal fluid, intraperitoneally or intrapeurally) or systemically (intravenously, intraarterially, intraportally or endocardially).

The nature and site of the disease and of the target cell to be transduced determine the following selection from the above-cited examples of promoter sequences and structural genes (for protein BCD or B'BCD):

a) Therapy of tumors

Promoters [component a)]:

- endothelial cell-specific or cell cycle-specific, or
- cell-nonspecific or muscle cell-specific and cell cycle-specific, or
- tumor cell-specific (solid tumors or leukemias)

Ligands for the following target cells [component b')]:

- proliferating endothelial cells, or
- stroma cells and muscle cells adjacent to the endothelial cell, or
- tumor cells or leukemia cells

Structural genes [component b)c)d)]:

- for coagulation-inducing factors for complement factors
- for angiogenesis inhibitors
- for cytostatic and cytotoxic proteins
- for inducers of inflammations, or
- for enzymes for activating precursors of cytostatic agents, for example for enzymes which cleave inactive precursor substances (prodrugs) into active cytostatic agents (drugs)

b) Therapy of autoimmune diseases and inflammations

Promoters [component a)]:
- endothelial cell-specific and cell cycle-specific, or
- macrophage-specific and/or lymphocyte-specific and/or cell cycle-specific, or
- synovial cell-specific and/or cell cycle-specific

Ligands for the following target cells [component b')]:
- proliferating endothelial cells, or
- macrophages and/or lymphocytes, or
- synovial cells

Structural genes [component b)c)d)]:
- for the therapy of antibody-mediated autoimmune diseases
- for inhibitors of cell proliferation, cytostatic or cytotoxic proteins, or
- enzymes for activating precursors of cytostatic agents, or
- for the therapy of arthritis

c) Therapy of damage to the nervous system

Promoters [component a)]:
- glia cell-specific, or

- endothelial cell-specific and cell cycle-specific, or
- nonspecific and cell cycle-specific

Ligands for the following target cells [component b')]:
- glia cells, or
- proliferating endothelial cells

Structural genes [component b)c)d)]:
- for neuronal growth factors, for example
- for cytokines and cytokine inhibitors which inhibit or neutralize the neurotoxic effect of TNFα.

d) Therapy of disturbances of the blood coagulation system and the blood circulation system

Promoters [component a)]:
- cell-nonspecific, or
- cell-nonspecific and cell cycle-specific, or
- specific for endothelial cells, smooth muscle cells or macrophages, or
- specific for endothelial cells, smooth muscle cells or macrophages and cell cycle-specific

Ligands for the following target cells [component b')]:
- endothelial cells
- proliferating endothelial cells
- somatic cells in the vicinity of endothelial cells and smooth muscle cells or macrophages

Structural genes [component b)c)d)]:
- for inhibiting coagulation or for promoting fibrinolysis.
- for angiogenesis factors
- for hypotensive peptides
- for an antiproliferative, cytostatic or

cytotoxic protein, or
- for an enzyme for cleaving precursors of cytostatic agents into cytostatic agents for inhibiting the proliferation of smooth muscle cells after damage to the endothelial layer, or
- for blood plasma proteins
  - C1 inactivator
  - serum cholinesterase
  - α1-antitrypsin

e) the therapy of chronic infectious diseases

Promoters [component a)]:
- virus-specific
- cell-specific, or
- virus-specific or cell-specific and cell cycle-specific

Ligands for the following target cell [component b')]:
- liver cell
- lymphocyte and/or macrophage
- epithelial cell, or
- endothelial cell

Structural genes [components b)c)d)], for example for
- a protein which exhibits cytostatic or cytotoxic effects.
- an enzyme which cleaves a precursor of an antiviral or cytotoxic substance into the active substance.
- for antiviral proteins, such as cytokines and growth factors which have an antiviral effect.

3.9 Preparation of the novel nucleic acid constructs

The nucleic acid constructs which are described here are prepared by linking together the individual components in accordance with standard molecular biological

methods.

The invention is clarified with the aid of the following examples:

4) Examples for the clarification of the invention

4.1) Preparation of a gene construct for an active compound which can be activated by prostate-specific antigen.

A therapeutic agent for treating prostate carcinoma metastases is prepared. Despite the surgical removal of a prostate which has become carcinomatous, metastases of the prostate carcinoma, which are currently to a large extent incurable and lead to the death of the patient, frequently occur. Prostate carcinoma metastases of this nature induce angiogenesis. Furthermore, prostate carcinoma metastases secrete a tissue-specific enzyme, i.e. prostate-specific antigen (PSA). According to the invention, a gene construct is prepared, which gene construct, when introduced into proliferating endothelial cells, leads to the expression of a modified coagulation factor FX. The modification consists in replacing, in the gene for the natural FX, the nucleotide sequence for the natural cleavage site (whose cleavage leads to FXa which is active in coagulation) with a nucleotide sequence for a PSA-specific cleavage site. As a result, the PSA which is secreted by prostate carcinoma metastases is able to specifically activate the modified FX which is secreted by proliferating endothelial cells in the vicinity of the metastases and thereby initiate coagulation, leading in turn to interruption of the blood supply to the metastasis and consequently to necrosis of the metastasis.

a) The gene construct for the PSA-activatable FX is prepared in accordance with a scheme which is

depicted in Figure 3.

The DNA sequences of the individual components are joined together in the 5' to 3' direction as follows:

a.1) Component a):
The promoter sequence of the cdc25C gene [nucleic acids: -290 to +121; Lucibello et al., EMBO J. 14, 132 (1995); Zwicker et al., Nucl. Acids Res. 23, 3822 (1995) and EMBO J. 14, 4514 (1995))

- the sequence GCCACC (Kozak, J. Cell Biol. 108, 229 (1989))

- the cDNA for the immunoglobulin signal peptide [nucleotide sequence ≤ 63 to ≥ 107; Riechmann et al., Nature 332, 323 (1988))

a.2) Component b)c)d):

- The cDNA for human FX (nucleotide sequence 1 to ≥ 1468) [Messier et al., Gene 99, 291 (1991)] in which amino acid 194 is mutated from Arg to Tyr.

The individual components of the construct are linked by way of suitable restriction sites which are concomitantly introduced at the termini of the different elements during PCR amplification. The linking is effected using enzymes which are specific for the restriction sites and which are known to the skilled person and DNA ligases. These enzymes can be obtained commercially.

The nucleotide construct which has been prepared in this way is cloned into puc 18/19 or Bluescript-derived plasmid vectors.

b) Expression in human embryonic kidney cells

Proliferating human embryonic kidney cells [HEK 293; Racchi et al., J. Biol. Chem. 268, 5735 (1993)] which are being maintained in culture are transfected with the described plasmid using the method known to the skilled person [Graham and van der Eb, Virol. 52, 456 (1973)].

The mutated factor X is purified from the supernatant from approx. 10^7 transfected HEK 293 cells [Watzke et al., J. Clin. Invest. 88, 1685 (1991)] and tested in a coagulation test for factor X with and without the addition of PSA. Purified PSA is obtained from Chemicon (Temecula, CA, USA).

In this test, the coagulation defect in human FX-deficient plasma is compensated for by functionally active FXa.

Unmutated (wild-type) FX (which is activated by Russel's viper venom) is employed as the positive control. A mock preparation from the supernatant from untransfected HEK 293 cells is used as a negative control in addition to the test mixture without PSA.

The coagulation activity of the mutated FX is measured using recalcification time (Seitz R et al., Int. J. Cancer 53:514-520, 1993). 100 µl of Fx-deficient plasma (Behring) are incubated, at 37° for 120 sec, with 100 µl of the FX preparation from the cell supernatant. The FX preparation contains PSA as activator. No PSA is added in the case of the negative control. FX (wild-type) and Russel's viper venom (RVV) is employed as the positive control. The coagulation reaction is augmented by adding 100 µl of 0.02 M $CaCl_2$ and determined in a coagulometer.

The following results are obtained:

The negative controls, in which coagulation was not activated, yielded a coagulation time of approx. 200 sec. By contrast, significantly reduced coagulation times of 50 sec. can be achieved using activated FX (mutated FX and PSA or wild-type FX and RVV).

It may be concluded, therefore, that the transduced HEK 293 cells express mutated FX which, in the presence of added PSA, compensates for the coagulation defect in FX-deficient plasma.

b) Expression in human endothelial cells

Human umbilical cord endothelial cells which are being maintained in culture are transfected with the described plasmid using the method known to the skilled person (Lucibello et al., EMBO J. 14, 132 (1995)).

In order to check cell cycle specificity, endothelial cells are synchronized in G0/G1 by removing methionine over a period of 48 hours (Nettlebeck et al., publication in preparation). The DNA content of the cells is determined in a fluorescence-activated cell sorter after staining with Hoechst 33258 (Lucibello et al., EMBO J. 14, 132 (1995)).

The expression of the gene construct is checked in the supernatant from the endothelial cells in analogy with the investigation carried out on HEK 293 cells.

The following results are obtained:

In contrast with mock preparations from the supernatant from untransfected endothelial cells, the protein which is expressed by transfected endothelial cells results in the coagulation defect in FX-deficient plasma being compensated.

A markedly higher concentration of mutated FX can be detected in the supernatant from proliferating, transduced endothelial cells (DNA > 2S) than in the supernatant from endothelial cells which are synchronized in G0/G1 (DNA = 2S).

Consequently the gene construct which has been described leads to cell cycle-dependent expression of the gene for the mutated FX in endothelial cells, and this mutated FX can be activated by PSA such that it brings about coagulation in FX-deficient plasma.

Legends to the Figures:

Figure 1: Diagrammatic depiction of a novel nucleic acid construct

Figure 2: Diagrammatic depiction of a novel nucleic acid construct which has been enlarged by adding component b'

Figure 3: Diagrammatic depiction of a gene construct for PSA-activatable factor X.

Patent claims

1. A gene construct which expresses a protein which is activated by enzymes which are released by cells, wherein the gene construct comprises the components

a) at least one promoter element
b) at least one DNA sequence for an active compound (protein B)
c) at least one DNA sequence for an amino acid sequence (part structure C) which can be cleaved specifically by an enzyme which is released from a mammalian cell
d) at least one DNA sequence for a peptide or protein (part structure D) which inhibits the activity of the active compound (protein B) by binding to the active compound (protein B) by way of the cleavable amino acid sequence (part structure C).

2. A gene construct as claimed in claim 1), wherein the enzymes are proteases.

3. A gene construct as claimed in one of claims 1 or 2, wherein the cells are tumor cells, leukemia cells, endothelial cell, macrophages, lymphocytes, muscle cells, epithelial cells, gliacells, synovial cells or virus-infected cells.

4. A gene construct as claimed in one of claims 1 to 3, wherein the gene construct is supplemented by the component b'), which constitutes at least one DNA sequence for a ligand (part structure B') which binds the active compound (protein B) to a target structure.

5. A gene construct as claimed in one of claims 1 to 4, wherein the part structure C can be cleaved by plasminogen activators, cathepsins, matrix metalloproteinases such as collagenases, stromelysins, metrilysins or gelatinases, elastases, prostate-specific

antigen or pancreatic trypsinogens.

6. A gene construct as claimed in one of claims 1 to 5, wherein the part structures B and D are parts of the natural precursors of protein active compounds, with the natural cleavage sequence which connects part structures B and D having been replaced by part structure C.

7. A gene construct as claimed in one of claims 1 to 6, wherein part structure D is the part structure of the natural precursor of a protein active compound.

8. A gene construct as claimed in one of claims 1 to 7, wherein part structure B (active compound protein B)

- activates the coagulation system
- inhibits the coagulation system
- activates fibrinolysis
- activates the complement system
- activates the kinin system
- is an enzyme which converts the inactive precursor of a pharmacological substance into the active substance, or
- is itself a pharmacologically active substance.

9. A gene construct as claimed in claim 8, wherein part structure B (active compound protein B) is

- a coagulation factor which is selected from thrombin, factor Va, factor VIIa, factor IXa, factor Xa, TF fragments which are active in coagulation, or factor XIIa
- thrombin which is mutated in the region of the Arg-Thr cleavage site (amino acid position 327/328)
- a fibrinolytic protein which is selected from urokinase, tPA or hybrids thereof
- a complement factor which is selected from CVF, C3b or cleavage products thereof
- an antithrombotic protein which is selected from

protein C, C-1S inhibitor, α1-antitrypsin, hirudin, AT-III, TFPI, PAI-1, PAI-2 or PAI-3

- a kallikrein
- a cytostatic, cytotoxic or inflammatory protein
- an antiangiogenic protein
- an immunomodulatory protein
- a protein having an antiinflammatory effect
- a protein which relieves damage to the nervous system
- a protein which inhibits or neutralizes the neurotoxic effect of TNFα
- a protein which stimulates angiogenesis
- a hypotensive protein, or
- an antiviral protein.
- a cytokine, an interferon, a tumor necrosis factor, oncostatin M or LIF
- a cytokine receptor, the part of a cytokine receptor which is external to the cell, or a cytokine antagonist
- a growth factor, a growth factor receptor or the part of a growth factor receptor which is external to the cell
- a chemokine
- angiostatin or platelet factor 4
- TIMP-1, TIMP-2 or TIMP-3
- a nitroreductase, a β-glucuronidase, a carboxypeptidase, a β-lactamase, a cytosine deaminase, a catalase, a peroxidase, a phosphatase or an oxidase, or
- kallikrein or an endothelial cell nitric oxide synthase.

10. A gene construct as claimed in one of claims 1 to 9, which comprises at least two identical or different components b)c)d) or b')b)c)d), which components are connected to each other by way of an internal ribosomal entry site (IRES).

11. A gene construct as claimed in one of claims 1 to

10, wherein component a) is a promoter sequence which can be activated in an unrestricted manner, a viral promoter sequence, a promoter sequence which can be activated metabolically, a promoter sequence which can be activated cell cycle-specifically, a promoter sequence which can be activated by tetracycline, or a promoter sequence which can be activated cell-specifically.

12. A gene construct as claimed in one of claims 1 to 11, wherein component a) constitutes a combination of at least two identical or different promoter sequences.

13. A gene construct as claimed in one of claims 10 to 11, wherein component a) is preferentially activated in endothelial cells, in cells in the vicinity of activated endothelial cells, in muscle cells, in leukemia cells, in tumor cells, in glia cells, in lymphocytes, in macrophages or in synovial cells.

14. A gene construct as claimed in one of claims 1 to 13, wherein component b') encodes a ligand (part structure B') which binds to a cell membrane receptor, to a cell membrane antigen or to an adhesion molecule which is located in the cell membrane or to the extracellular matrix.

15. A gene construct as claimed in one of claims 1 to 14, wherein the ligand is

- a growth factor, a cytokine, an interferon, a tumor necrosis factor or a chemokine or the receptor-binding part sequence of these ligands
- a peptide hormone
- angiotensin, kinin or folic acid
- an adhesion molecule or the part sequence of the adhesion molecule which binds to the corresponding adhesion molecule or to the extracellular matrix
- the extracellular part of an Fc receptor

- an antibody molecule, the epitope-binding part of an antibody molecule, a single chain Fv fragment or a recombinant Fv fragment which is specific for a cell membrane antigen or for an antigen on the extracellular matrix, or
- a glycoprotein of a virus which has a tropism for selected cells or a part sequence of the glycoprotein which binds to these cells.

16. A gene construct as claimed in claim 14, wherein the ligand preferentially binds to endothelial cells, to activated endothelial cells, to proliferating endothelial cells, to muscle cells, to proliferating endothelial cells, to muscle cells, to macrophages, to lymphocytes, to synovial cells, to inflammatory cells, to virus-infected cells, to liver cells, to bronchial epithelial cells, to glia cells, to leukemia cells or to tumor cells.

17. A gene construct as claimed in one of claims 1 to 12, wherein component b' encodes a ligand (part structure B') which constitutes the transmembrane domain of a receptor or of a viral glycoprotein or which is a glycophospholipid anchor.

18. A nucleic acid construct as claimed in one of claims 1 to 17, which is inserted into a plasmid or into a viral vector.

19. A nucleic acid construct as claimed in claim 18 for the therapy of tumors, leukemias, autoimmune diseases, inflammations, damage to the nervous system, disturbances of blood coagulation and the blood circulation, and of infectious diseases.

20. The use of a nucleic acid construct as claimed in claim 1 to 19 for preparing a drug for administering locally (e.g. onto the skin, nasally, orally, gastrointestinally, intrabronchially, intravesicularly, intra-

vaginally, into the uterus, subcutaneously, intramuscularly, periarticularly, intraarticularly, into the cerebrospinal fluid, into the brain tissue, into the tissue of the spinal cord, into wounds intraperitoneally or intrapleurally) or systemically (e.g. intravenously, intraarterially, intraportally or endocardially) for the prophylaxis and/or therapy of of tumors, leukemias, autoimmune diseases, inflammations, damage to the nervous system, disturbances of blood coagulation and the blood circulation, and of infectious diseases.

21. A process for preparing a gene construct as claimed in one of claims 1 to 19, which comprises linking the components which contain the gene construct to each other using standard molecular biological methods.

Abstract of the disclosure

Gene constructs for active substances which can be activated by proteases

The invention relates to a gene construct which expresses a protein which is activated by enzymes which are released by cells, wherein the gene construct comprises the components

a) at least one promoter element
b) at least one DNA sequence for an active compound (protein B)
c) at least one DNA sequence for an amino acid sequence (part structure C) which can be cleaved specifically by an enzyme which is released by a mammalian cell
d) at least one DNA sequence for a peptide or protein (part structure D) which inhibits the activity of the active compound (protein B) by binding to the active compound (protein B) by way of the cleavable amino acid sequence (part structure C);

and to the use of the nucleic acid construct for preparing a drug for treating diseases.

Figure 1

Structural gene

Component a)	Component b)	Component c)	Component d)
Promoter element	DNA sequence for an active compound (protein B)	DNA sequence for an amino acid sequence (part structure C) which can be cleaved by tumor proteases	DNA sequence for a peptide (part structure D) which inhibits the active compound (protein B) when bound to part structure C

↓

Protein BCD, which is encoded by the structural gene

Figure 2

Structural gene

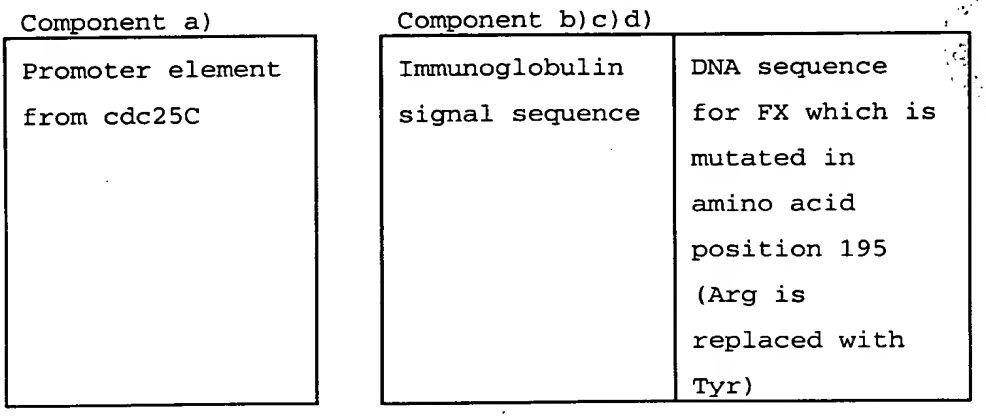
Component a)	Component b')	Component b)	Component c)	Component d)
Promoter element	DNA sequence for a ligand (part	DNA sequence for an active compound	DNA sequence for an amino acid sequence (part structure C)	DNA sequence for a peptide (part structure D)

structure B')	(protein B)	which can be cleaved by tumor proteases	which inhibits the active compound (protein B) when bound to part structure C

↓

Protein B'BCD, which is encoded by the structural gene

Figure 3


Component a)
Promoter element from cdc25C
Component b)c)d)
Immunoglobulin signal sequence
DNA sequence for FX which is mutated in amino acid position 195 (Arg is replaced with Tyr)
5' 5' 5'
3' 3' 3'